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# Bone Marrow Treatment of Mice Lethally Irradiated with Gamma-Rays under High Dose Rate. (II)

## Effect of Homologous Bone Marrow

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Dd/s strain mice, male and female, were lethally irradiated with gamma-rays under high dose rate and were treated with homologous na2 strain mouse bone marrow. Although a 14 day survival rate was fairly good, 30 day survival was generally low except for experiment No. 2 using female mice. However, most of the mice which survived for the initial 30 days in experiment No. 2 died during the subsequent 30 days.

## INTRODUCTION

The effect of isologous bone marrow on lethally irradiated mice was described in Report I<sup>21)</sup>. Isologous bone marrow, however, can be obtained only in mice or a few other animal species in which there are inbred strains. The results of animal experiments with isologous bone marrow treatment are applicable to humans only in the case of uniovular twins. Thus from a practical point of view, bone marrow treatment of an irradiated recipient which belongs to the same species as the donor but differs genetically is much more important than isologous bone marrow treatment. The following is the result of homologous bone marrow treatment in mice lethally irradiated with gamma-rays under high dose rate.

## MATERIALS AND METHODS

Inbred dd/s strain mice, male and female, were used as irradiated bone marrow recipients. Inbred na2 strain mice were used as bone marrow donors. Both the donors and recipients were 2 to  $2\frac{1}{2}$  months old. The method of obtaining bone marrow suspensions and the conditions of irradiation were described in Report I<sup>21)</sup>. Mice in experiment No. 1 and 2 were irradiated at place B and others at place A.

## RESULTS

### 1) Survival Rate

Survival rates of mice in several experiments are shown in Table 1. The

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Table 1. Survival rate of gamma-irradiated mice treated with homologous bone marrow.

Experiment No.	Sex	Dose rate (r/min.)	Dose (r)	No. of nucleated cells injected $\times 10^6$	Survival No. of living/No. of irradiated						% Survival	
					at 7 days	14	21	30	60	90	21 days	90
1	♂	318	900	1.7~5.6	T 8/13 C 9/11	5/12 1/11	2/12 1/11	2/12 1/11	1/12 1/11	1/12 1/11	17 9	8 9
2	♀	318	900	3.2~3.6	T 13/13 C 7/7	11/12 1/7	11/12 1/7	9/11 1/7	3/10 1/7	3/10 1/7	92 14	30 14
3	♂	3500	875	7.6	T 9/16 C 11/12	4/16 0/12	3/16	2/16	1/16	1/16	19 0	6 0
4	♂	3470	752	6.0	T 13/14 C 8/8	7/13 0/8	4/13	1/13	1/13	1/13	31 0	8 0
5	♀	3390	848	7.8	T 6/8 C 2/4	4/8 1/4	2/8 1/4	0/8 1/4	0/4		25 25	0 0

T : Treated    C : Control

difference in survival rate in experiment No. 1 between the treated mice and the controls is not significant, but that in experiment No. 2 is significant with 99% probability. Twenty one day survival rates of the irradiated and treated mice were higher than the controls in most of the experiments, but generally lower than those in Report 1. The survival rate in the longer periods

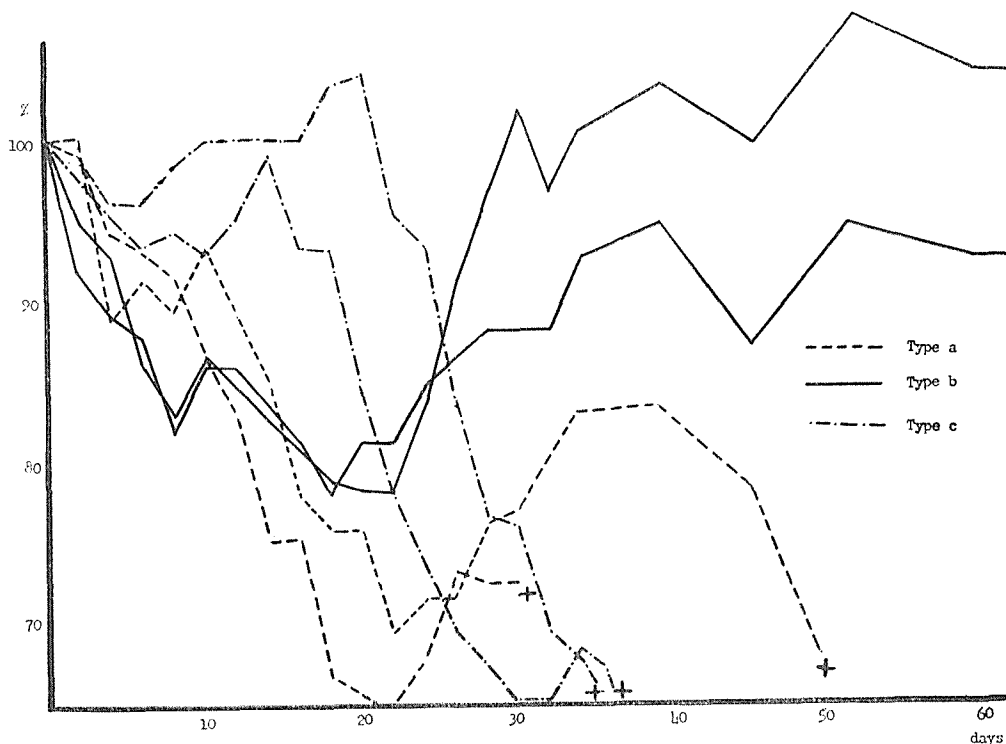


Fig. 1. Individual weight change in gamma-irradiated mice treated with homologous Bone marrow. (Experiment No. 2)

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decreased further. The survival rate of the treated mice in experiment No. 2 was 92% at 21 days, but decreased to 30% at 60 days. The main causes of death in mice dying between 21st and 60th day were pneumonia, sepsis, and intestinal hemorrhage just as in those dying before the 21st day. In a long term observation, the last mouse in the treated group in experiment No. 1 died at 205 days, and in experiment No. 2 three treated ones died at 120, 180 and 274 days and one of the controls at 109 days. Further, the last mouse in experiments No. 3 and 4 died at 117 days and 96 days, respectively. No apparent cause of death was found in most of the mice dying after 90 days.

### 2) Body Weight Changes

The mean weight changes do not show the tendency of weight change in individual animal, if mice which are gaining weight and those which are losing weight are included in a same group. Individual weight changes of six

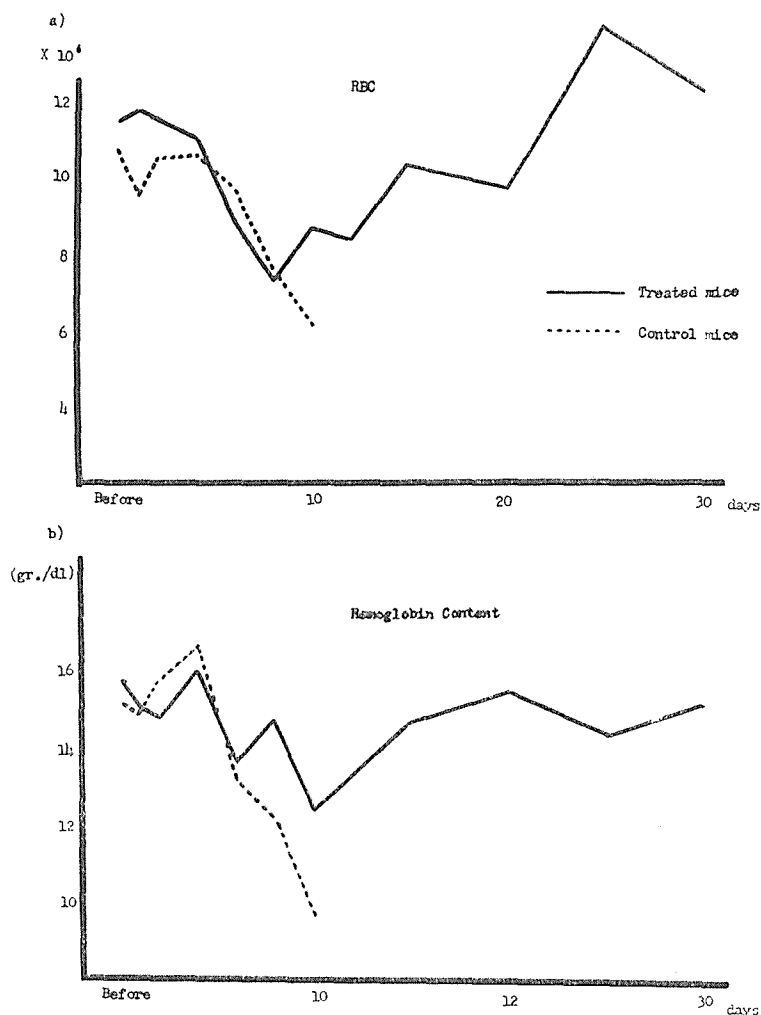


Fig. 2. RBC and Hb content in gamma-irradiated mice treated with homologous bone marrow.

representative animals in experiment No. 2 are shown in Fig. 1. From this figure three types of weight changes are noted. a) Mice which showed a rapid and continuous decrease of body weight which went too far to be compensated by a terminal recovery. b) Mice which showed a moderate degree of weight loss followed by its gradual increase beginning around the 20th day. c) Mice which showed a rather slight degree of weight loss followed by a tendency to increase by the 20th day. Then the body weight started to decrease rapidly and the mice died between 30 and 60 days. The weight change of type-a was approximately the same as that of the controls, showing no favorable effects of bone marrow treatment. The weight change of type-b was approximately the same as that of mice treated with isologous bone marrow, though there was a longer period of initial body weight decrease in the former. The weight

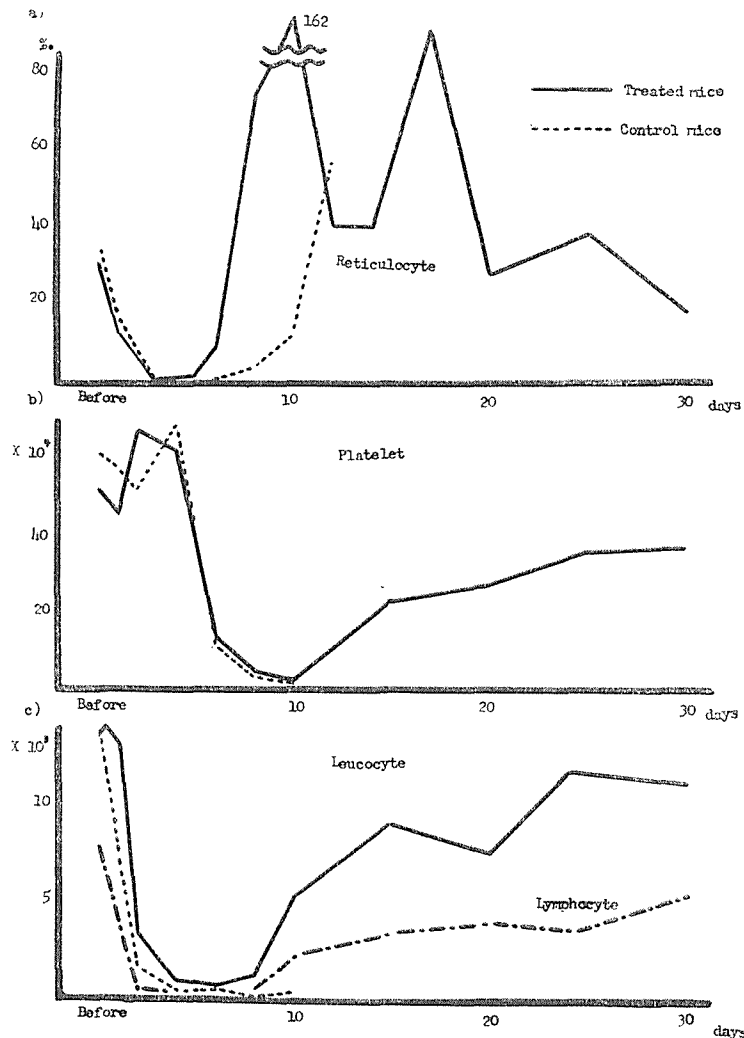


Fig. 3. Reticulocyte, platelet, and leucocyte count in gamma-irradiated mice treated with homologous bone marrow.

change of type-c was seen exclusively with homologous bone marrow treatment.

### 3) Hematological findings (Figs. 2 and 3)

Changes of peripheral erythrocyte count, leucocyte count, hemoglobin content, reticulocyte count, and platelet count after gamma-irradiation and homologous bone marrow treatment were essentially the same as those in mice gamma-irradiated and treated with isologous bone marrow (21). Most of the mice dying between 21 and 60 days had almost normal blood cell count except for a few with a moderate decrease in leucocyte count.

### 4) Histological findings

Histological findings in the bone marrow and the spleen during the period immediately after irradiation and the beginning of recovery were the same as with isologous bone marrow treatment: complete wasting within 48 hours after irradiation followed by spotty regeneration of the bone marrow and early phase of the recovery of the red pulp of the spleen beginning at 4 or 5 days. A rapid recovery to normal cellularity followed. All of the mice dying between 30 and 60 days had normal cellularity of their bone marrow except for one which had only 25% of normal. There was no difference in the speed of regeneration of the red pulp of the spleen between homologously and isologously treated mice, but the recovery of the white pulp in the former showed much delay beginning no earlier than the ninth week. There was no evidence of regeneration of the lymphnodes during 21 day observation period in both groups. The regeneration of the inguinal lymphnodes was observed for the first time at 41 days and not earlier in the treated group. In no animal did the thymus show evidence of regeneration before the 40th day, much slower than in those irradiated and treated with isologous bone marrow. As for changes in other organs, submucosal edema of the small intestine, and focal necrosis or abscess of the liver were occasionally found in the treated mice.

## DISCUSSION

The fact that mice fatally irradiated and treated with homologous bone marrow survived for the first 3 to 4 weeks with survival rate almost equal to that of isologously treated mice, and that they died in the subsequent 60 days was reported by Congdon *et al.*<sup>3)</sup> and others. A kind of immune reaction has been thought to be involved in this phenomenon in which the mice treated with homologous bone marrow lose weight despite normal food intake leading to death. The speed of recovery of the bone marrow was the same as that in mice treated with isologous bone marrow, but there were differences in other organs, viz., a delay in regeneration of the white pulp of the spleen, thymus and lymphnode. The exact cause of this delay in regeneration of the lymphatic organs is not known, but may be due either to the direct effect of the immune reaction or indirectly to metabolic effects caused by the immune reaction. Congdon *et al.*<sup>4)</sup> stated that extreme atrophy of the white pulp of the spleen and lymphnodes was due to exhaustion in the presence of large

numbers of foreign cells which became strong stimuli to the host. They also stated that the thymus recovered temporarily followed by a simple atrophy, and that this secondary atrophy of the thymus might be related to the emaciated condition of the mice. In the present study temporary regeneration of the thymus was not observed, however.

There are two theories concerning the antigen and antibody involved in the immune reaction described above. One is that donor (graft) cells become an antigen and sensitize the host tissue, and the other is that the host tissue becomes an antigen, sensitizing the graft. Should an immune reaction occur by the former process, it is called a host-to-graft reaction, and if by the latter, a graft-to-host reaction. As experimental data in favor of the former theory, Gengozian *et al.*<sup>6)</sup> observed the antibody response of lethally irradiated mice treated with either mouse or rat bone marrow. a) Irradiated mice treated with isologous mouse marrow responded at 30 days differently to sheep RBC antigen and rat RBC antigen. If donor tissue is responsible for antibody formation, then the response to both antigens should be the same as seen in normal mice. b) The response to both antigens recovered gradually, and this might mean a recovery of the irradiation damaged antibody-forming tissue of the host. c) In irradiated mice treated with heterologous bone marrow, there was no response to isologous mouse RBC antigen. If antibody producing cells were those of the donor (rat), there should be a response to mouse RBC. d) The response to sheep RBC was weak. Since sheep RBC antigens are foreign to both mouse and rat antibody-forming tissues, the response to this antigen should be additive. Makinodan<sup>9)</sup> was unable to find rat serum protein in the serum of rat marrow protected mice by the serum-agar method. There has been no direct evidence for transplantation of non-granulocytes. When mice were pre-sensitized to foreign bone marrow cells before irradiation, a higher per centage of mice died earlier than those without pre-sensitization<sup>10)</sup>.

As for the experimental data in favor of the latter, graft-to-host reaction theory, Trentin<sup>13)</sup> stated that the essentially normal blood count of mice treated with homologous marrow at the time of their late death and the long duration of skin homograft without sloughing did not accord with the former theory. Uphoff<sup>15)</sup> observed that delayed death did not take place when lethally irradiated parent strain mice were treated with F<sub>1</sub> hybrid mouse marrow, but did in the reverse situation. Since F<sub>1</sub> hybrid has all the antigenic components of a parent strain, the former does not form antibodies to the latter. On the other hand the former has antigenic components not shared with the latter, which should form antibodies to the former. Thus Uphoff's experiment gave support to the graft-to-host reaction theory. He also extended the study of the F<sub>1</sub> hybrid effect in making a F<sub>1</sub> hybrid of two parent strains which were different only in histocompatibility-2 locus of chromosome to each other<sup>16)</sup>. If the graft-to-host reaction is the case, using the fetal tissue as a donor should preclude immune reactions; this was confirmed by Uphoff<sup>17)</sup> but not by Congdon *et al.*<sup>4)</sup> Though Congdon was unable to find rat serum protein in rat marrow protected mice,

Weyzen *et al.*<sup>20)</sup> reported that rat gamma globulin was demonstrated by precipitation with anti-rat serum and by micro-immuno-electrophoresis.

However, there are several studies and opinions against the graft-to-host reaction theory. Fox<sup>5)</sup> stated that one dominant gene one antigen concept which formed the basis of Uphoff's opinion<sup>15,16)</sup> could not be applicable in all situations.

Even if rat gamma globulin was found in rat marrow protected mice there is no evidence to prove that it is an antibody to the mouse tissue. There have been increasing evidence to support the opinion that antibody-forming cells are plasma cells<sup>21)</sup>. There are usually no plasma cells in the normal mouse bone marrow<sup>22)</sup>. Even if they are found in the bone marrow of the mouse they may be mostly mature in type as in the case of human bone marrow and do not appear to proliferate readily upon stimulation<sup>23)</sup>. So it is doubtful that enough plasma cells proliferate to make enough antibody to kill the host. The mechanism of delayed death in irradiated animals protected by homologous bone marrow needs to be studied further.

In the present study, survival of irradiated mice protected by homologous bone marrow was generally less than that of mice protected by isologous bone marrow except for the female mice in experiment No. 2. In Report I also, female mice responded better to isologous bone marrow treatment. Although Abrams<sup>1)</sup> observed no sex difference in X-irradiation sensitivity, Watanabe<sup>19)</sup> found female mice less sensitive than male mice and Langendorf *et al.*<sup>8)</sup> observed also that female mice were less sensitive but became sensitive when they were castrated. As for the ability to respond to bone marrow protection Urso *et al.*<sup>18)</sup> observed the tendency for male mice to recover with relatively smaller dose of bone marrow but the difference was small. Other workers usually did not comment on the sex difference in this aspect and there was presumably no difference. Estrogen has been known to reduce radiation mortality<sup>12)</sup> and pre-treatment with estrogen supplements the effect of bone marrow treatment after irradiation<sup>11)</sup>. Thus it is not surprising to obtain better survival rate in females after irradiation and bone marrow protection.

In the present study, a thirty day survival rate of mice was comparatively low except for experiment No. 2. However, a 14 day survival rate was fairly good. The antibody-forming tissue of the host may have recovered earlier than in experiments using the same dose of X-rays because of the low RBE of gamma-rays. Gengozian *et al.*<sup>7)</sup> reported that sublethally irradiated mice died early with homologous bone marrow treatment. Even Trentin, who supports the graft-to-host reaction theory in the case of lethal irradiation, agrees with the host-to-graft reaction theory in the case of high sublethal irradiation<sup>14)</sup>.

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irradiation facility.

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